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Neuroscience Letters 292 (2000) 171–174

Neuroscience Letters

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Suppression of experimental allergic encephalomyelitis is specific to the frequency and intensity of nocturnally applied, intermittent magnetic fields in rats

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Received 19 July 2000; received in revised form 18 August 2000; accepted 18 August 2000

Abstract

Female Lewis rats ($n = 72$) were inoculated with an emulsion of spinal cord and complete Freund's adjuvant. They were then exposed for approximately 6 min every hour between midnight and 08:00 h for 2 weeks to either 7 or 40 Hz amplitude-modulated magnetic fields whose temporal pattern was designed to simulate a (geomagnetic) storm sudden commencement. The peak strengths of the fields averaged between either 30–50 nT (low intensity) or 500 nT (high intensity). Rats exposed to the 7 Hz, low intensity magnetic fields displayed significantly less severe overt signs of experimental allergic encephalomyelitis than rats exposed to either of the two intensities of the 40 Hz fields, the high intensity 7 Hz field, or the reference (<10 nT) condition. The latter groups did not differ significantly from each other. Predicted severity based upon the numbers of foci of infiltrations of lymphocytes within the brains of the rats also demonstrated the ameliorating effects of the low intensity, 7 Hz exposures. These results suggest very specific characteristics of complex, weak magnetic fields within the sleeping environment could affect the symptoms of autoimmunity. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Magnetic fields; Experimental allergic encephalomyelitis; Rat; Multiple sclerosis; Nocturnal exposures; NanoTesla range; Amplitude modulations

Associations between perturbations in the geomagnetic field and changes in the activity of living systems are well documented [1,2,9]. For several years we have been pursuing the biobehavioral effects from exposures to complex temporal patterns of low intensity (<500 nT, <5 mG) magnetic fields that simulate components of the onset of a geomagnetic storm [10]. According to Mayaud [7] the rise times for most storm sudden commencements during the last 100 years have been about 5 or 6 min. The changes in intensity of the most active component of the geomagnetic field have ranged from a few nanoTeslas to 100 nT with median values between 15 and 50 nT.

We have selected 5–6 min patterns of amplitude-modulated 7 Hz square waves as potential candidates to influence the electromagnetic activity within the brain. This frequency, although the wave form is more sinusoidal, is also prominent for hundreds of seconds or longer in the power spectra [11] of the resonances generated within the spherical cavity between the earth and the lower ionosphere and within the normal electrical profiles of aggregates of limbic neurons. Whole body exposures to these patterns have reduced the severity of the clinical signs of rats inoculated to elicit experimental allergic encephalomyelitis (EAE) [8]. EAE has been considered a dysfunction similar to multiple sclerosis (MS) in human populations [12].

Similar to the effects of magnetic fields upon melatonin levels [5,6] reported by other researchers [4], the suppression of overt signs of EAE was strongest when the experimental field, that simulated geomagnetic activity, was presented during the late scotophase (midnight to 08:00 h). The present study was designed to discern the specificity of the immunosuppressive effect of nocturnal exposures to 7 Hz, square wave magnetic fields with maximum amplitude modulations of 50 nT by comparing the severity of EAE symptoms after rats had been exposed 6 min/h for 8 h during the scotophase for 2 weeks to either 7 or 40 Hz magnetic fields whose peak amplitude modulations were either 50 or 500 nT. We selected a 40 Hz magnetic field so we could begin to discern the specificity of the ameliorating effects,

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PII: S0304-3940(00)01454-3

predicted for the 7 Hz fields, upon EAE. The 500 nT amplitude modulation was included as a variable to discern if the effect was augmented by simple intensity.

Seventy-two female Lewis rats (aged 16–20 weeks of age), obtained from Charles River, Inc (Montreal, Quebec), were employed as subjects in separate blocks of experiments. All rats were injected in both hindfoot pads with female Lewis rat spinal cord emulsified in Freund's complete adjuvant (40 mg/kg; 0.1 cc injection/footpad). Immediately following inoculation, the rats were weighed and placed two per cage. Each of two plastic cages ($32 \times 36 \times 45$ cm) was surrounded along its major axis by a coil (30 ohms) of wire (AWG 20). The two coils were connected to a custom built output for a Model EU 81A Heath–Schlumberger function generator [10]. Two other identical cages were maintained 30 cm below the two coils and housed the reference groups.

When the field was activated by the Commodore computer, the intensity increased in sequential steps seven times during the first 3 min and 20 s and seven times during the last 1 min and 42 s. The duration of each plateau was 14 s during the first cluster and 7 s during the last cluster. The time required to change to each increment of intensity was 2 μs. The pattern is shown in Fig. 1. The total exposure time was 5 min and 50 s every hour from 00:00 to 07:06 h. Room temperature was automatically controlled within one degree of 20°C. The light–dark cycle was 12:12 h with the onset time of 08:00 h.

There were nine blocks of experiments, each separated by between 1 and 6 months, that were conducted between July 1996 and December 1998. Blocks consisted of four rats exposed within the two coils and four rats housed within the reference area below the coils. The long axes of the four cages were oriented in a N–S direction. The background (primarily geomagnetic) steady-state magnetic field within the exposure areas, with no experimental fields activated, ranged between 30 000 and 38 000 nT (resultant values) according to a FM300 Vector Magnetometer (MacIntrye Electronics, Herndon, VA). Lighting within the four cages was supplied by overhead fluorescent lamps and was between 700 and 800 lux (Gossen–Panlux meter).

During non-consecutive blocks either a square-wave 7 Hz field or square-wave 40 Hz field was generated through the coils. For any given block the average peak intensity within the coils during the 6 min pattern was either about 50 nT (±10%) or 500 nT (±10%). The presentation of the fields was verified by the computer log that recorded both the time and status of the coils. The presence and intensities of the fields were calibrated before the beginning of each block and measured (verified) by the second author every second night with a Metex M-3800 digital multimeter and sensor (Electric Field Measurement, W. Stockbridge, MA 01266). During the application of the 500 nT, 7 and 40 Hz fields within the solenoids, stray (extended) fields within the two reference cages averaged between 25 and 30 nT.

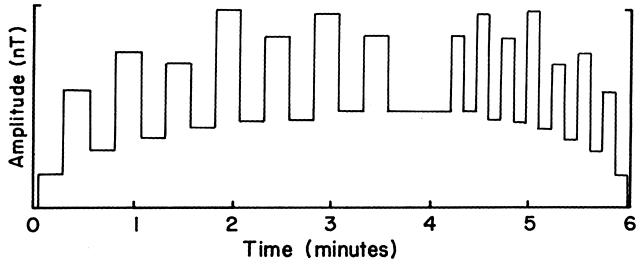

Fig. 1. Temporal pattern of the amplitude modulations of the applied 7 and 40 Hz fields. The maximum amplitude was either 50 or 500 nT within the coils for different blocks of experiments.

The occurrence of these stray fields allowed a test to eliminate the possibility that any magnetic field effects might be artifacts of the presence of the coils around the exposure cages. When the 50 nT fields were generated within the coils, the intensities within the lower (reference) cages never fluctuated and were less than 10 nT. The potential penetration of intermittent magnetic fields from the adjacent room through the walls into the experimental cages was eliminated by prohibiting the use of electronic equipment within these areas.

The exposure time for each block was 15 nights. On post-inoculation days 13–15, all the rats were examined and rated on a scale of 0–5 [8] for clinical symptoms (0, normal; 1, tail atonia; 2, ataxia; 3, partial hindlimb paresis; 4, complete hindlimb paresis and incontinence; 5, complete paresis and death). The rating was completed by the first author who was not familiar with either: (1) the condition of each block of experiments, or (2) the occurrence of extended fields into the adjacent cages when the 500 nT fields were generated within the coils. The symptoms were qualitatively obvious and showed an inter-rater reliability greater than 0.9. On day 15, all rats were weighed and decapitated. Our pilot studies had indicated that after this time (until 25 days post-inoculation) the symptoms of non-exposed rats improved and the symptoms of rats exposed to 7 Hz, low intensity fields either remained asymptomatic or improved.

The brains were fixed in an ethanol-formalin-acetic acid solution and processed. Coronal, 10 μm sections were taken every 100 μm of brain between the area postrema and anterior commissure and stained with toluidine blue 0. The numbers of foci of lymphocytic infiltrations, as defined previously [3] for all of the 50 major structures within this volume were counted at 400× magnification. Because of the skewness of numbers of foci in most of the structures, square-root transformations were completed for subsequent computations. The means for the EAE scores for each of the 3 days examined as well as the relative weight loss ((final weight-initial weight)/final weight) were calculated. All statistics analyses involved SPSS software. To detect the possible influence of geomagnetic activity, daily global aa values were obtained from the Geomagnetic Indices Bulletin, Boulder, CO.

Because there were no statistically significant differences between the direct and indirect (stray fields) low intensity

groups following the 7 Hz exposures or the direct and indirect low intensity groups following the 40 Hz exposures, they were combined into a low intensity 7 Hz group (25–50 nT) and a low intensity (30–50 nT) 40 Hz group. Two way analysis of variance of the severity of the symptoms as a function of days and the five treatments showed a statistically significant treatment difference ($F(4, 67) = 12.09$, $P < 0.001$; eta-squared = 0.41, i.e. 41% of the variance explained). The means and standard errors of the mean for the EAE scores are shown in the upper panel of Fig. 2.

There was a general worsening of the symptoms over post-inoculation days 13–15 ($F(2, 134) = 13.97$, $P < 0.001$; eta-squared = 0.16) as well as a statistically significant interaction between treatments and days ($F(8, 136) = 2.04$, $P < 0.05$; eta-squared = 0.11). Post-hoc analysis (Tukey, $P < 0.05$) indicated that whereas the rats exposed to 40 Hz fields (regardless of intensity) continued to worsen over the observation period the rats exposed to the 7 Hz fields (regardless of intensity) displayed a relatively greater suppression of overt symptoms. There were no significant differences between the groups for relative changes in body weight during the treatment ($F(4, 67) = 2.08$, $P > 0.05$).

The results of a multiple regression analysis indicated the severity of the overt expression of EAE was correlated 0.69 ($F(6, 66) = 9.90$, $P < 0.0001$; adjusted $r^2 = 0.43$) with the square root of the numbers of foci of infiltrations of lymphocytes within six (of the possible 50) structures. These structures, according to the order of entry into the equation (standardized beta, partial regression coefficients in parentheses) were the thalamus (0.57), trochlear complex (−0.41), inferior colliculus (0.31), mamilliary bodies (−0.33), lateral hypothalamus (0.44) and lateral lemniscus (−0.37). We extracted this equation to discern if the pattern of infiltration of mononuclear cells within multiple structures of the brain, most correlated with the clinical signs, also showed the effects of the 7 Hz, 50 nT exposures. The means and standard errors of the mean for the square root of numbers of foci per section for the first structure (the thalamus, the mean for 44, bilateral intrathalalmic divisions) to enter the equation for the groups were: control 0.32 (0.04), low 7 Hz 0.14 (0.04), high 7 Hz 0.28 (0.15), low 40 Hz 0.29 (0.07) and high 40 Hz 0.38 (0.07).

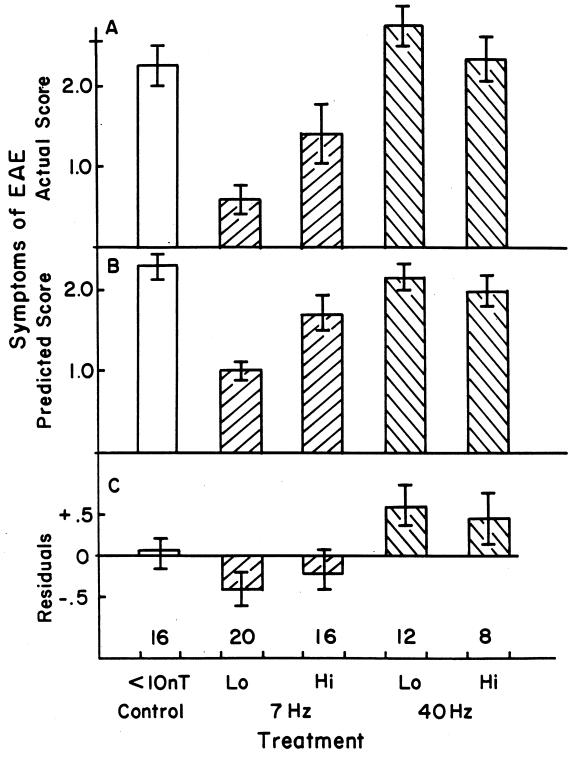

Fig. 2. Means and standard errors of the mean for the scores for the overt symptoms of experimental allergic encephalomyelitis, the predicted scores from infiltrations of lymphocytes in specific brain regions, and the residuals, for various treatments. Values above the *x*-axis refer to the numbers of rats per condition.

The means and standard errors of the mean for the predicted scores derived from the equation composed of the square root of the numbers of lymphocytic foci within the six brain structures are also shown in Fig. 2 for each group. One way analyses of variance indicated statistically significant group differences (all d.f.'s = 4,67) for the predicted scores ($F = 6.39$, $P < 0.001$) and the residuals ($F = 3.62$, $P < 0.01$). Post-hoc (Tukey, $P < 0.05$) tests indicated, as expected, that the major source of the differences for the predicted scores was due to the much less intense symptoms of EAE for the rats exposed to the 7 Hz, low intensity magnetic fields compared to the other four groups that did not differ significantly from each other. We suspect the group differences for the means of the residuals between the low intensity 7 and 40 Hz fields were related to changes in the spinal cord that we did not assess.

The results of this experiment indicated that the reduction of overt deficits associated with EAE following exposures to nocturnal, amplitude-modulated magnetic fields was specific to a frequency of 7 Hz and intensities between approximately 25 and 50 nT. Rats exposed to 7 Hz fields with larger amplitude modulations or to 40 Hz fields with modulations within the 30–50 nT range or the 500 nT range did not display the reduction of the symptoms and did not differ from non-treated controls.

Because the rater of the overt symptoms was not aware of the experimental condition during each block or the presence of the stray fields (25–30 nT) within the reference cages when the coils were activated with 500 nT variations, the likelihood of subtle bias was minimal. In addition, the predicted values of EAE based upon the numbers of foci of infiltrations of lymphocytes determined histologically also showed that the major source of the group differences was due to the rats exposed to the 7 Hz, low intensity fields.

Whether or not exposures of less than 2 weeks and less than eight times per night to low intensity 7 Hz fields would be sufficient to evoke similar effects remains to be measured. The mean, daily global aa values ranged between 13 and 23 nT during the different experimental

blocks of this study. There was no significant correlation (rho $= -0.08$) between the severity of the symptoms per block and these geomagnetic values.

We recently found an attenuation of clinical signs (rho $= -0.60, P = 0.05$) in eight control (sham-field) blocks if sudden storm commencements had occurred during the 15 days of exposure. However the quantitative shift in scores associated with this correlation was small and ranged (means and standard deviations in parentheses) between 2.6 (0.7) and 2.0 (0.2). The possibility that the temporal configurations of magnetic fields could help ameliorate the symptoms associated with human autoimmune disorders must still be explored.

Thanks to Stan Koren for technical assistance.

[1] Burch, J.B., Reif, J.S. and Yost, M.G., Geomagnetic disturbances are associated with reduced nocturnal excretion of a melatonin metabolite in humans, Neurosci. Lett., 266 (1999) 209–212.
[2] Bureau, Y.R.J. and Persinger, M.A., Decreased latencies for lithium/pilocarpine-induced limbic seizure in rats when daily average geomagnetic activity exceeds 20 nT, Neurosci. Lett., 192 (1995) 142–144.
[3] Cook, L.L. and Persinger, M.A., Infiltration of lymphocytes in the limbic brain following stimulation of subclinical cellular immunity and low dosages of lithium and a cholinergic agent, Toxicol. Lett., 109 (1999) 77–85.
[4] Lerchl, A., Honaka, K.O. and Reiter, R.J., Pineal gland 'magnetosensitivity' to static magnetic fields is a consequence of induced electric currents (eddy currents), J. Pineal Res., 10 (1991) 109–116.
[5] Maestroni, G.J.M., Conti, A. and Pierpaoli, W., Role of the pineal gland in immunity: circadian synthesis and release of melatonin modulates the antibody response and antagonized the immunosuppressive effect of corticosterone, J. Neuroimmunol., 13 (1986) 19–30.
[6] Maestroni, G.J.M., The immunoneuroendocrine role of melatonin, J. Pineal Res., 14 (1993) 1–10.
[7] Mayaud, P.N., Analysis of storm sudden commencements for the years 1868-1967, J. Geophys. Res., 80 (1975) 111–122.
[8] Missaghi, B., Richards, P.M. and Persinger, M.A., Severity of experimental allergic encephalomyelitis in rats depends upon the temporal contiguity between limbic seizures and inoculation, Pharmcol. Biochem. Behav., 43 (1992) 1081–1086.
[9] Persinger, M.A., Increased geomagnetic activity and the occurrences of bereavement hallucinations: evidence for melatonin-mediated microseizuring of the temporal lobe? Neurosci. Lett., 88 (1988) 271–274.
[10] Persinger, M.A., Cook, L.L. and Koren, S.A., Suppression of experimental allergic encephalomyelitis in rats exposed six minutes per hour nocturnally to amplitude-modulated (60 nT) 7 Hz magnetic fields, Int. J. Neurosci., 100 (2000) 107–116.
[11] Persinger, M.A., Ludwig, H.W. and Ossenkopp, K.-P., Psychophysiological effects of extremely low frequency electromagnetic fields: a review, Percept. Mot. Skills, 36 (1973) 1131–1159.
[12] Simmons, R.D., Bermarad, C.C., Kerlo del Rosbo, N. and Carnegie, P.R., Criteria for an adequate explanation of typical clinical signs of EAE in rodents, In E.C. Alvord, M.W. Kies and A.J. Suckling, (Eds.), Experimental Allergic Encephalomyelitis: A Useful Model for Multiple Sclerosis, Liss, New York, 1984, pp. 23–30.